

Clean Set of All Pending Claims

**February 12, 2003**

1. A microarray comprising a surface silanized with a silane in toluene in the absence of acetone or an alcohol, and a target molecule, wherein the target molecule is attached to the surface via the silane.
2. A microarray comprising a surface silanized with a silane in toluene in the absence of acetone or an alcohol, a linker, and a target molecule, wherein the target molecule is attached to the surface via the linker.
3. The microarray of claim 2, wherein the target molecule is a polynucleotide.
4. The microarray of claim 3, wherein the polynucleotide is selected from the group consisting of an oligonucleotide, DNA, amplified DNA, cDNA, single stranded DNA, double stranded DNA, PNA, RNA, and mRNA.
5. The microarray of claim 4, wherein the polynucleotide has a length in the range of about 3 bp to 10 kb.
6. The microarray of claim 5, wherein the length is in the range of about 100 bp to 5 kb.
7. The microarray of claim 6, wherein the length is in the range of about 0.3 kb to 3 kb.
8. The microarray of claim 7, wherein the length is in the range of about 0.5 kb to 2 kb.
9. The microarray of claim 4, wherein the polynucleotide is an oligonucleotide and the oligonucleotide is 25-1000 bp, 25-500, 30-200, and 50-100 bp in length.
10. The microarray of claim 2, wherein the target molecule is a polynucleotide and comprises an amine.
11. The microarray of claim 10, wherein the amine group is a primary amine.

12. The microarray of claim 11, wherein the primary amine is at the 5' end of the polynucleotide.
13. The microarray of claim 11, wherein the primary amine is attached at the 5' end of the polynucleotide via a linker, wherein the linker comprises one or more monomers of 1-20 carbon atoms, and wherein the monomer comprises a linear chain of carbons or a ring or both.
14. The microarray of claim 12, wherein the polynucleotide is prepared by extending a nucleic acid primer comprising a primary amine at its 5' end.
15. The microarray of claim 2, wherein the substrate surface is selected from the group consisting of polymeric materials, glasses, ceramics, natural fibers, nylon, nitrocellulose, silicones, metals, and composites thereof.
16. The microarray of claim 15, wherein the substrate surface is planar.
17. The microarray of claim 15, wherein the substrate is in a form selected from the group consisting of threads, sheets, films, gels, membranes, beads, and plates.
18. The microarray of claim 15, wherein the substrate surface is glass.
19. The microarray of claim 18, wherein the substrate is a glass slide.
20. The microarray of claim 2, wherein the target molecule is attached after contacting the target molecule with the surface by a technique selected from the group consisting of printing, capillary device contact printing, microfluidic channel printing, deposition on a mask, and electrochemical-based printing.
21. The microarray of claim 20, wherein the target molecule is unmodified prior to the contacting.
22. The microarray of claim 21, wherein the target molecule is modified to comprise an amine prior to the contacting.

23. The microarray of claim 22, wherein the amine is a primary amine.
24. The microarray of claim 23, wherein the target molecule is a polynucleotide and the primary amine is at the 5' end of the polynucleotide.
25. A microarray prepared by a method comprising:
  - (a) providing a multifunctional linker reagent comprising two or more reactive groups, wherein a first reactive group reacts with a functional group of a microarray substrate and a second reactive group reacts with a target molecule;
  - (b) activating the substrate surface for immobilizing the target molecule, by silanizing the surface with a silane in toluene in the absence of acetone or an alcohol, wherein the silane comprises a functionality reactive with the multifunctional linker reagent, and wherein the activating further comprises immobilizing the multifunctional linker reagent on the silanized surface by attaching the multifunctional linker reagent to the silane via the first reactive group of the linker reagent and a reactive group of the silane;
  - (c) providing a solution comprising a target molecule having one or more functional groups reactive with the second reactive group of the immobilized multifunctional linker reagent;
  - (d) attaching the target molecule to the substrate surface by contacting the target molecule with the activated substrate surface under conditions that promote attachment of the target molecule to the immobilized multifunctional linker reagent.
26. The microarray of claim 25, wherein the target molecule is a polynucleotide, and wherein the contacting of step (d) is carried out by spotting the polynucleotide on an activated substrate surface.
27. The microarray of claim 26, wherein the polynucleotide is unmodified.
28. The microarray of claim 26, wherein the polynucleotide is modified with an amine group.
29. The microarray of claim 28, wherein the amine group is a primary amine at the 5' end of the polynucleotide.

30. The microarray of claim 26, wherein the polynucleotide is spotted on the surface at a concentration in the range of approximately 0.1  $\mu\text{g}/\mu\text{l}$  to and including approximately 3  $\mu\text{g}/\mu\text{l}$ .
31. The microarray of claim 25, wherein the attaching of step (d) occurs in a pH range from pH 6 to and including pH 10.
32. The microarray of claim 31, wherein the pH range is from pH 6.5 to and including pH 9.7.
33. The microarray of claim 32, wherein the pH range is from pH 7 to and including pH 9.4.
34. The microarray of claim 33, wherein the pH is 9.3.
35. The microarray of claim 25, wherein the attaching is allowed to occur for a time period from 1 minute to and including 24 hours.
36. The microarray of claim 35, wherein the time period is from 1 - 24 hours.
37. The microarray of claim 36, wherein the time period is from 5-18 hours.
38. The microarray of claim 37, wherein the time period is from 10-16 hours.
39. The microarray of claim 38, wherein the time period is from 12-14 hours.
40. The microarray of claim 25, wherein the method of preparing the microarray further comprises, after step (d), blocking unreacted reactive groups.